Hormonal regulation of leptin and leptin receptor expression in porcine subcutaneous adipose tissue

T. G. Ramsay and M. P. Richards

J Anim Sci 2004. 82:3486-3492.

Hormonal regulation of leptin and leptin receptor expression in porcine subcutaneous adipose tissue[1,2]

T. G. Ramsay[3] and M. P. Richards

Growth Biology Laboratory, USDA-ARS, Beltsville, MD 20705

ABSTRACT: The current study was performed to examine the response of the leptin gene to hormonal stimuli in porcine adipose tissue from finishing pigs. Yorkshire gilts (approximately 150 kg BW) were used in this study. Tissue from four to six pigs was used per experiment. Dorsal subcutaneous adipose tissue samples were acquired, and adipose tissue explants (approximately 100 mg) were prepared using sterile technique. Tissue slices were transferred to 12-well tissue culture plates containing 1 mL of Media 199 with 25 m*M* HEPES, 0.5% BSA, pH 7.4, and various hormone supplements. Triplicate tissue slices were incubated with either basal medium or hormone-supplemented media in a tissue culture incubator at 37°C with 95% air:5% CO_2. Hormones included insulin (100 n*M*), dexamethasone (1 μ*M*), porcine GH, 100 ng/mL), triiodothyronine (T_3, 10 n*M*), porcine leptin (100 ng/mL), or IGF-I (250 ng/mL). Following incubation for 24 h, tissue samples from the incubations were blotted and transferred to microfuge tubes, frozen in liquid N, and stored at –80°C before analysis for gene mRNA abundance by reverse-transcription PCR and subsequent quantification of transcripts by capillary electrophoresis with laser-induced fluorescence detection. Media from the incubations were collected in microfuge vials and stored at –20°C before analysis for leptin content by RIA. Insulin was required to maintain tissue and mRNA integrity; therefore, insulin was included in all incubations. The combination of insulin and dexamethasone stimulated leptin secretion into the medium by 60% ($P < 0.05$; n = 6). Porcine GH inhibited insulin induced leptin secretion by 25% ($P < 0.05$; n = 6). Dexamethasone in combination with insulin produced a 22% increase in leptin mRNA abundance relative to insulin ($P < 0.05$; n = 4), and T_3 stimulated a 28% increase in insulin-induced leptin mRNA abundance ($P < 0.05$; n = 4). Leptin receptor mRNA abundance was decreased by 25% with the combination of insulin and dexamethasone, relative to insulin-treated adipose tissue slices ($P < 0.05$; n = 4). Porcine GH decreased leptin receptor mRNA abundance by 17% ($P < 0.05$; n = 6). These data suggest that leptin secretion is a regulated phenomenon and that posttranslational processing may be significant. Alternatively, transport and exocytosis of leptin containing vesicles in the pig adipocyte may be quite complicated, which could account for the differences in observed mRNA abundance and protein secretion.

Key Words: Adipose, Leptin, Leptin Receptor, Swine

 J. Anim. Sci. 2004. 82:3486–3492

Introduction

Leptin is a peptide secreted from the adipose tissue that may play an important role in suppressing appetite in swine through actions at the hypothalamus (Barb et al., 1998). Leptin mRNA has been detected in pig adipose tissue (Ramsay et al., 1998; Leininger et al., 2000), with expression exclusive to the adipocyte (Chen et al., 1997). Several studies have been published that demonstrate hormonal regulation of porcine leptin mRNA levels in vitro (Chen et al., 1997, 1998; Ramsay and White, 2000). Those studies have used adipocytes differentiated in vitro from neonatal adipose tissue, rather than adipocytes obtained from the pig. The current study was performed to examine the response of the leptin gene in porcine adipose tissue to hormonal stimuli.

Changes in leptin mRNA level were correlated with adiposity (Leininger et al., 2000). Differences in tissue leptin mRNA abundance were associated with differences in serum leptin concentrations (Ramsay et al., 1998). Secretion of leptin into the bloodstream was con-

[1]Mention of a trade name, vendor, proprietary product or specific equipment is not a guarantee or a warranty by the U.S. Department of Agriculture and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

[2]The authors thank M. Stoll for her assistance with the RNA extractions and reverse transcription PCR. We also acknowledge S. Poch for providing training and assistance with the transcription-PCR procedures. The authors also thank J. Dobrinksy, Biotechnology and Germplasm Laboratory, USDA-Beltsville, for providing animals used in this study.

[3]Correspondence: BARC-East, Bldg. 200, Rm. 207 (phone: 301-504-5958; fax: 301-504-8623, e-mail: tramsay@anri.barc.usda.gov).

Received December 31, 2003.

Accepted August 9, 2004.

firmed by western analysis (Ramsay et al., 1998) and by RIA (Barb et al., 2001). However, the relationship between changes in leptin mRNA abundance and leptin protein secretion from the adipose tissue in swine is unknown and was the purpose of the current study.

Materials and Methods

Yorkshire gilts (approximately 150 kg BW) were used for these experiments, with four to six gilts used per experiment. Gilts were provided water and a corn-soybean diet (18.3% CP, 3,059 kcal/kg of ME; as fed) ad libitum. Animals of this size were intentionally used because of the enlarged adipose mass containing a higher proportion of large adipocytes than present in the adipose tissue from smaller swine (Etherton et al., 1981). Adipose tissue with large adipocytes has been shown to express higher levels of leptin than tissue containing small adipocytes (Van Harmelen et al., 1998), thereby enhancing the ability to detect secreted leptin. Dorsal s.c. adipose tissue samples from between the second and fourth thoracic vertebrae were acquired following slaughter by electrical stunning and exsanguination according to procedures approved by the Institutional Area Animal Use and Care Committee. The middle s.c. adipose tissue was dissected free of the outer s.c. adipose to reduce variability in hormone responses. Dissected adipose tissue was diced into 1- × 4-cm strips and placed in Hanks buffer (37°C, pH 7.4) in screw-capped polypropylene Erlenmeyer flasks for transport to the laboratory, approximately 2 min from the abattoir.

In the laboratory, adipose tissue strips were placed in fresh Hank's buffer (37°C, pH 7.4). Adipose tissue strips were dissected clean of any extraneous muscle tissue and further separated into 1-cm cubes in a laminar-flow hood. Adipose tissue explants (approximately 100 mg) were prepared by slicing tissue cubes with a Stadie-Riggs microtome. Tissue slices (400 μm thickness) were rinsed twice with fresh Hanks buffer (37°C, pH 7.4), blotted free of excess liquid, and weighed. Tissue slices were then transferred to 12-well tissue culture plates containing 1 mL of media 199 with 25 m*M* HEPES, 0.5% BSA, pH 7.4, and the various hormone supplements of interest. Triplicate tissue slices were incubated with either basal medium or hormone supplemented media in a tissue culture incubator at 37°C with 95% air:5% CO_2.

Hormones included 100 n*M* insulin, 100 ng/mL porcine GH, 1 μ*M* dexamethasone, 10 n*M* triiodothyronine, 250 ng/mL of IGI-1, or 100 ng/mL porcine leptin. Sterile hormone solutions were prepared and frozen in vials before initiation of the experiment. Porcine insulin (Sigma-Aldrich, St. Louis, MO) was solubilized in 0.001 N HCl. Dexamethasone (Sigma-Aldrich) was solubilized in ethanol. Porcine GH (USDA-pGH-B-1, Beltsville, MD) was diluted in 25 m*M* sodium bicarbonate buffer, pH 9.4 (Na_2CO_3,$NaHCO_3$). Triiodothyronine (**T_3**; Sigma-Aldrich) was prepared in 0.01 *N* NaOH. Human recombinant IGF-I (Sigma-Aldrich) was solubilized with 0.1% acetic acid. Recombinant porcine leptin was acquired from A. Gertler (Raver et al., 2000) and solubilized in PBS. Following initial solubilization, all hormones were subsequently diluted in 0.5% BSA in saline, aliquoted, and frozen. Individual hormone aliquots were thawed for each day of use and diluted in incubation medium to the appropriate concentration. Control incubations in basal medium included a similar amount of vehicle to exclude the vehicle as a variable. The selected hormone concentrations were determined in preliminary experiments or based on concentrations described in the literature to affect leptin/leptin receptor expression for insulin (Ramsay and White, 2000), dexamethasone (Murakami et al., 1995), GH (Chen et al., 1998), IGF-I (Morash et al., 2000), T_3 (Yoshida et al., 1997), and leptin (Wang et al., 1999).

Following 24 h of incubation, tissue samples from these incubations were blotted and transferred to microfuge tubes with subsequent freezing in liquid N and storage at –80°C before analysis for mRNA abundance. Media from incubations was collected in microfuge vials and stored at –20°C before analysis for leptin content. Media samples were centrifuged (10,000 × *g*) for 10 min at 4°C and duplicate 100-μL aliquots were collected for measurement of media leptin content by RIA (Multispecies RIA kit, Linco, St. Charles, MO). Human leptin standards were replaced with recombinant pig leptin standards. Recombinant pig leptin was obtained from A. Gertler (Raver et al., 2000). Cross-reactivity for the recombinant porcine leptin in the multispecies leptin RIA was 58%. The intraassay and interassay CV were 8.32 and 16.3%.

Gene Expression Analyses by Reverse-Transcription Polymerase Chain Reaction

Total RNA was isolated using TRI reagent according to the manufacturer's protocol (Sigma-Aldrich). Integrity of RNA was assessed via agarose gel electrophoresis and RNA concentration and purity were determined spectrophotometrically for absorbance of light at 260 and 280 nm. Reverse-transcription (**RT**) PCR (20 μL) consisted of 1 μg of total RNA, 50 U of SuperScript II reverse transcriptase (Invitrogen/Life Technologies, Carlsbad, CA), 40 U of an RNAse inhibitor (Invitrogen/Life Technologies), 0.5 mmol/L dNTP, and 100 ng of random hexamer primers. Polymerase chain reactions were performed in 25 μL containing 20 mmol/L Tris-HCl, pH 8.4, 50 mmol/L KCl, 1.0 μL of the RT reaction, 1.0 U of Platinum Taq DNA polymerase (Hot Start; Invitrogen/Life Technologies), 0.2 mmol/L dNTP, 2.0 mmol/L Mg^{2+} (Invitrogen/Life Technologies), 10 pmol each of the leptin and leptin receptor specific primers, and 10 pmol of an appropriate mixture of primers and competimers specific for 18S rRNA (QuantumRNA Universal 18S Internal Standard; Ambion, Inc; Austin, TX). Thermal cycling parameters were as follows: 1 cycle 94°C for 2 min, followed by 30 cycles, 94°C for 30 s,

58°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 8 min.

The following primers were used for generating 348-bp PCR products corresponding to a portion of the pig leptin coding sequence: 5′-TGACACCAAAACCCTCATCA-3′ (forward), 5′-GCCACCACCTCTGTGGAGTA-3′ (reverse). The primers for the leptin receptor were used to generate a 393-bp product corresponding to the extracellular domain, thus encompassing the total leptin receptor population: 5′-ACTGGAGCACCCCCTTTACT-3′ (forward), 5′-TGGTTGACCATCTGCAAGTC-3′ (reverse).

The RT-PCR techniques were optimized in a series of preliminary experiments. The two-step duplex RT-PCR for leptin + 18S or leptin receptor + 18S were optimized for linearity (exponential amplification) from >25 to <35 cycles under the conditions described above.

Capillary Electrophoresis with Laser-Induced Fluorescence Detection

Aliquots (2 μL) of RT-PCR samples were diluted 1:100 with deionized water before capillary electrophoresis with laser-induced fluorescence detection (**CE/LIF**). A detailed description and validation of the CE/LIF technique used in this study for quantitative analysis of RT-PCR products was reported previously (Richards and Poch, 2002). Briefly, a P/ACE MDQ CE instrument (Beckman Coulter, Fullerton, CA) equipped with an argon ion LIF detector was used. Capillaries were 75 μm i.d. × 32 cm μSIL-DNA (Agilent Technologies, Folsom, CA). EnhanCE dye (Beckman Coulter) was added to the DNA separation buffer (Sigma-Aldrich) to a final concentration of 0.5 g/L. Samples were loaded by electrokinetic injection at 3.5 kV for 5 s and run in reverse polarity at 8.1 kV for 5 min. Integrated peak area for the PCR products separated by CE was calculated using P/ACE MDQ software (Beckman Coulter).

Quantification of Gene Expression

For this study, estimates of the abundance of mRNA were determined as the ratio of integrated peak area for each individual gene PCR product relative to that of a coamplified 18S internal standard (QuantumRNA Universal 18S Internal Standard; Ambion, Inc, Austin, TX). Standardization with a coamplified 18S internal standard will correct for sample handling. Thus, a corrected or relative value for the target gene sequence is produced for each sample. Although CE/LIF is a quantitative procedure for measuring RNA products (Richards and Poch, 2002), RT-PCR can best be described as semi-quantitative (Joyce, 2002; O'Connell, 2002). Relative values are presented as the mean ± SEM of four to six individual determinations.

Statistical Analyses

The experimental model for these experiments was a completely randomized design. Data were normalized relative to insulin medium (100 n*M*) to account for culture-to-culture variation. Data were analyzed by one-way AOV using SigmaStat software (SPSS Science, Chicago, IL). Mean separation was analyzed using Student-Newman-Keuls test. Means were defined as significantly different at $P < 0.05$.

Results

Leptin Secretion

Leptin secretion occurred during a 24-h exposure to the hormones and hormone combinations of interest. Basal secretion of leptin varied among the tissue cultures, ranging from 21 to 38 ng/mL for each 100 mg of tissue (29.7 ± 2.8; n = 6). Originally, the data were to be expressed relative to the basal medium; however, degradation of the mRNA was detected in this control group incubated with basal medium, indicating tissues incubated without a hormone supplement were deteriorating. Therefore, the data was expressed relative to the insulin treatment group wherein media leptin levels ranged from 26 to 39 $ng \cdot mL^{-1} \cdot 100\ mg\ tissue^{-1}$ (31.9 ± 3.2; n = 6), which did not differ significantly from the basal secretion rate (29.7 ± 2.8 $ng \cdot mL^{-1} \cdot 100$ mg of $tissue^{-1}$; $P = 0.56$; n = 6). As a result of this variability between tissue cultures, data were expressed relative to leptin secretion in insulin treated cultures for each animal.

In the first experiment, dexamethasone (1 μ*M*) was tested in combination with insulin for its efficacy in stimulation of leptin secretion (Figure 1). The combination of insulin and dexamethasone stimulated leptin secretion into the medium by 60% ($P < 0.001$). In a second experiment, porcine growth hormone addition to insulin-containing medium inhibited leptin secretion by 25% ($P < 0.01$) relative to incubations containing insulin. Triiodothyronine (10 n*M*) had no effect in combination with insulin on leptin secretion from adipose tissue slices (92.7 ± 10% of control; $P = 0.55$). Addition of IGF-I (250 ng/mL medium) in combination with insulin to the incubation medium did not affect leptin secretion (83.7 ± 11% of control; $P = 0.39$).

Leptin Gene Expression

Following the experiments examining the regulation of leptin secretion, further experiments were performed to evaluate hormonal regulation of leptin gene expression using tissue samples from additional pigs. Leptin mRNA was normalized to 18S rRNA, and then data were expressed relative to tissue cultures incubated with insulin containing medium to account for the culture-to-culture variation.

The combination of insulin with dexamethasone produced a 22% increase in leptin mRNA abundance relative to insulin (Figure 2; $P < 0.01$; n = 4). Incubation of tissue cultures with porcine growth hormone and insulin for 24 h had no effect on leptin mRNA abundance

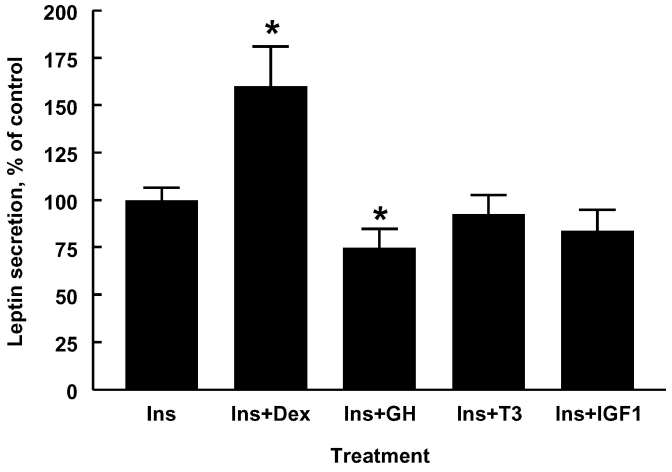

Figure 1. Relative percentage of leptin secretion in response to hormone exposure. Primary cultures of porcine adipose tissue were incubated with insulin (Ins, 100 n*M*), the combinations of insulin and dexamethasone (Dex, 1 μ*M*), insulin and porcine growth hormone (GH, 100 ng/mL), insulin and triiodothyronine (T3, 10 n*M*), or insulin and IGF-I (250 ng/mL) for 24 h, followed by collection of culture media for analysis of leptin content by RIA. Data are expressed relative to cultures incubated with insulin. Asterisks indicate that means differ ($P < 0.05$) from treatment with insulin (n = 6).

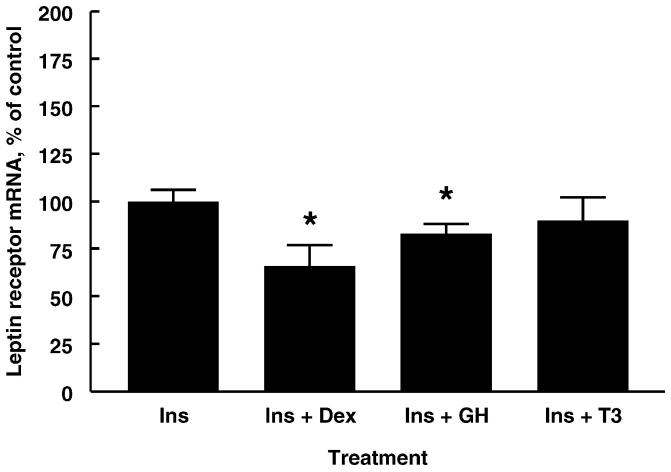

Figure 3. Relative abundance of leptin receptor mRNA in response to hormone exposure. Primary cultures of porcine adipose tissue were incubated with insulin (Ins, 100 n*M*; n = 6), the combinations of insulin and dexamethasone (Dex, 1 μ*M*; n = 4), insulin and porcine growth hormone (GH, 100 ng/mL, n = 6), or insulin and triiodothyronine (T3, 10 n*M*; n = 4) for 24 h, followed by extraction for total RNA and subsequent reverse-transcription PCR analysis for abundance of leptin receptor mRNA. Data are expressed relative to cultures incubated with insulin. Asterisks indicate that means differ ($P < 0.05$) from treatment with insulin.

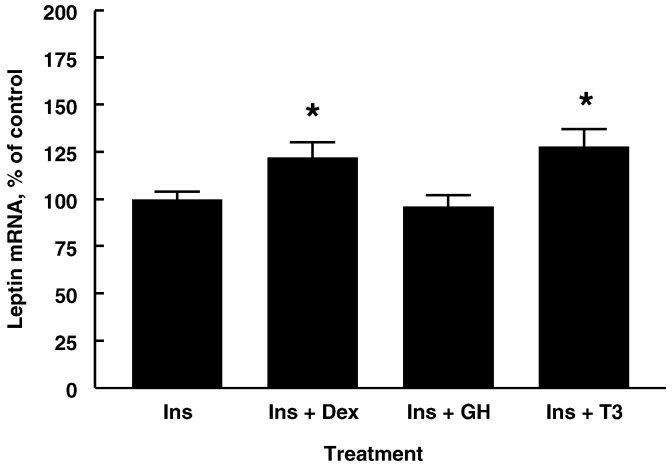

Figure 2. Relative abundance of leptin mRNA in response to hormone exposure. Primary cultures of porcine adipose tissue were incubated with insulin (Ins, 100 n*M*; n = 6), the combinations of insulin and dexamethasone (Dex, 1 μ*M*; n = 4), insulin and porcine growth hormone (GH, 100 ng/mL; n = 6), or insulin and triiodothyronine (T3, 10 n*M*; n = 4) for 24 h, followed by extraction for total RNA and subsequent reverse-transcription PCR analysis for abundance of leptin mRNA. Data are expressed relative to cultures incubated with insulin. Asterisks indicate that means differ ($P < 0.05$) from treatment with insulin.

(96.3 ± 6% of control; $P = 1.00$; n = 6), relative to cultures incubated with insulin alone. Tissue slices incubated with T_3 and insulin expressed leptin mRNA at a 28% higher level than slices incubated with insulin ($P < 0.01$; n = 4). Leptin in combination with insulin and dexamethasone produced a similar change in leptin mRNA level as insulin in combination with dexamethasone (108 ± 7% of insulin + dexamethasone; $P = 0.38$; n = 6; data not presented).

Leptin Receptor mRNA Abundance

Following 24 h of incubation, insulin in combination with dexamethasone produced a 25% decrease in leptin receptor mRNA abundance (Figure 3; $P < 0.03$; n = 4). Growth hormone inhibited insulin induced leptin receptor mRNA abundance by 17% ($P < 0.001$; n = 6). The combination of insulin and T_3 produced a similar effect on leptin receptor mRNA abundance as insulin alone (88.9 ± 11% of control; $P = 0.209$; n = 4). The addition of leptin in combination with insulin and dexamethasone had no effect on the abundance of leptin receptor mRNA (99.3 ± 9% of insulin + dexamethasone; $P = 0.710$; n = 6; data not presented).

Discussion

Adipose tissue is the primary source of leptin secreted into the bloodstream (Harris, 2000). This secretion is

through both regulated constitutive secretory pathways involving intracellular vesicle transport (Bradley et al., 2001). Hormonal regulation of leptin secretion has been demonstrated for both human and rodent adipose tissue (Guerre-Millo, 2002). Although regulation of leptin gene expression has been evaluated in newly differentiated pig adipocyte cell cultures (Chen et al., 1998), regulation of the secretion of porcine leptin in vitro has not been previously examined.

Insulin has been reported to stimulate leptin secretion from human and rodent adipocytes at concentrations as low as 0.16 n*M* (Mueller et al., 1998; Russell et al., 1998). The current study could not replicate these results with a concentration of 100 n*M* insulin. This may be the consequence of species variation due to the relative insulin resistance of the pig adipocyte compared with human or rodent adipocyte (Mersmann, 1989).

Because the insulin treatment group was used to make all comparisons of mRNA abundance, we cannot discern from the results of this study whether insulin stimulates or inhibits leptin gene expression, as it served as the control. However, previous research using pig adipocytes formed in vitro (Chen et al., 1998; Ramsay and White, 2000) and human adipose tissue (Russel et al., 1998) demonstrated that insulin can stimulate leptin expression, although the results were not usually evident at 24 h of incubation, but only after longer periods of time (e.g., 72 to 96 h).

Glucocorticoids have been previously demonstrated to induce leptin mRNA abundance and protein secretion from human (Halleux et al., 1998; Russell et al., 1998) and rodent adipose tissues (Murakami et al., 1995; Slieker et al., 1996). The increase in leptin mRNA steady-state level may be due in part to enhanced transcription, probably through binding of the steroid-receptor complex to the glucocorticoid response element in the regulatory region of the leptin gene (Gong et al., 1996). In the current study, the combination of insulin and dexamethasone stimulated leptin gene expression and protein secretion. Kanu et al. (2003) reported that insulin is required for a dexamethasone stimulation of leptin secretion from isolated subcutaneous adipocytes, whereas Halleux et al. (1998) indicated that insulin decreases dexamethasone-induced increases in leptin mRNA abundance and protein secretion from omental adipocytes. These conflicting results may be the consequence of regional differences in adipose tissue or methodology and demonstrate the need for examination of leptin regulation in the different adipose tissues of the pig.

The majority of research on the porcine leptin receptor has focused primarily on detection of the receptor rather than regulation of the receptor (Czaja et al., 2002). Lin et al. (2003) have recently reported that GHRH inhibited leptin receptor expression in the porcine pituitary in vitro. Unfortunately, few studies on the regulation of leptin receptor gene expression in the adipocyte have been performed. The data from the current study indicate that dexamethasone can inhibit total leptin receptor gene expression. Smith and Waddell (2002) have previously reported that dexamethasone can inhibit leptin receptor expression in the placenta, although no mechanism was defined. The inhibition of leptin receptor expression by dexamethasone, while stimulating leptin gene expression and protein secretion, might imply a downregulation of the receptor in the presence of elevated leptin protein levels, although this is speculation without examination of the leptin receptor binding and turnover.

Porcine GH inhibited insulin-induced leptin secretion, indicating some species variation in the regulation of leptin secretion. Growth hormone promotes insulin resistance in swine adipose tissue (Walton and Etherton, 1986). This may play a significant role in the observed inhibition of insulin induced leptin secretion because GH seems to not have a direct effect on the secretion of leptin as suggested by studies with mouse adipocytes (Fain et al., 1999; Lee et al., 2001). The inhibition of leptin secretion in the current study agrees with experiments in humans demonstrating an inverse relationship between plasma GH and plasma leptin concentrations (Florkowski et al., 1996; Fors et al., 1999). Although these in vivo experiments did not measure insulin, they cannot exclude the possible interaction of growth hormone with insulin mediated leptin secretion.

In vivo GH administration has been demonstrated to inhibit leptin gene expression by 56% in swine adipose tissue (Spurlock et al., 1998). In vitro, GH can inhibit the insulin-induced expression of leptin in bovine adipose tissue (Houseknecht et al., 2000) or porcine adipocytes differentiated in culture (Chen et al., 1998). In the current study, we did not observe an attenuation of the response to insulin when GH was added to the medium. The various responses among the reports suggest that species-specific or age-related responses to GH by the leptin gene exist. The data from the leptin secretion experiment in the current study indicate that GH inhibits leptin secretion, yet no change in leptin mRNA level was detected. These data would imply that posttranslational processing of leptin may be significant in the pig adipocyte. Alternatively, transport and exocytosis of leptin containing vesicles in the pig adipocyte may be quite complicated. Little is known concerning the mechanisms regulating the transport and exocytosis of these vesicles (Bradley et al., 2001). The disagreement between the leptin mRNA and protein secretion response to GH demonstrates the risk of interpreting mRNA data as indicative of an endocrine response to a stimuli, when it is only indicative of the mRNA.

Growth hormone was able to inhibit the insulin-induced expression of the leptin receptor. No specific effect of GH on the leptin receptor has been reported. This inhibitory effect may be the indirect through GH antagonism of insulin action in swine adipose tissue, as previously reported (Walton and Etherton, 1986).

The relative role of changes in the level of leptin receptor mRNA to either number or activity of functional leptin receptors has not been elucidated. There is a critical need for examination of leptin receptor kinetics as the potential significance of leptin receptor mRNA data has not been confirmed.

Many of the effects of GH have now been attributed to IGF-I through paracrine induction. Porcine GH has been demonstrated to produce a rapid increase in IGF-I expression in swine adipose tissue (Ramsay et al., 1995). In the current study, IGF-I did not alter leptin secretion into the medium, unlike GH, which inhibited leptin secretion by 25%. Isozaki et al. (1999) reported lower serum leptin concentrations and higher IGF-I levels in acromegalics than in normal subjects, suggesting that GH directly interacts with adipose tissue to decrease leptin expression. However, a significant positive correlation between serum leptin and serum IGF-I has been reported for normal human subjects, but was not associated with IGF-I regulation of leptin synthesis or secretion (Gomez et al., 2003). These studies support the hypothesis that GH is acting directly on the adipocyte and not through IGF-I to regulate leptin secretion.

Fain and Bahouth (1998) reported that T_3 treatment of adipose tissue explants from hypothyroid rats produces an increase in leptin secretion and elevated leptin mRNA levels when used in combination with insulin and dexamethasone. In the current study, T_3 had no effect on leptin secretion from porcine adipose tissue explants in the presence of insulin, but T_3 increased leptin mRNA abundance by 28%. Treatment with T_3 alone has been shown to increase leptin mRNA and secreted leptin levels in cultures of 3T3-L1 adipocytes (Yoshida et al., 1997), to have no effect in human omental adipose tissue explants (Menendez et al., 2001), or to inhibit leptin secretion and gene expression in human s.c. adipose tissue (Kristensen et al., 1999). The true function of T_3 in regulation of leptin gene expression and secretion may be difficult to determine, as the variability between studies suggests that the response depends on the entire hormonal milieu, time of incubation, tissue source, and species. Alternatively, variable conditions for culture among the studies might contribute to the differences in the T_3 response.

Implications

Leptin is a hormone produced by pig adipose tissue that can affect feeding behavior, animal health, and reproduction. Studies in pigs have demonstrated that leptin can decrease feed intake. This experiment was designed to determine whether the production and release of porcine leptin is a regulated phenomenon. Secondly, this study attempted to determine whether expression of leptin and its receptor could be manipulated by hormonal mechanisms in mature animals. The data demonstrate that leptin secretion can be both stimulated and inhibited. Secondly, expression of the leptin gene and the leptin receptor gene are responsive in adult swine. These results suggest the potential to manipulate the efficiency of feeding behavior in finishing animals at a time when changes in body composition can be critical for improving profitability.

Literature Cited

Barb, C. R., J. B. Barrett, R. R. Kraeling, and G. B. Rampacek. 2001. Serum leptin concentrations, luteinizing hormone and growth hormone secretion during feed and metabolic fuel restriction in the prepuberal gilt. Domest. Anim. Endocrinol. 20:47–63.

Barb, C. R., X. Yan, M. J. Azain, R. R. Kraeling, G. B. Rampacek, and T. G. Ramsay. 1998. Recombinant porcine leptin reduces feed intake and stimulates growth hormone secretion in swine. Domest. Anim. Endocrinol. 15:77–86.

Bradley, R. L., K. A. Cleveland, and B. Cheatham. 2001. The adipocyte as a secretory organ: mechanisms of vesicle transport and secretory pathways. Recent Prog. Horm. Res. 56:329–358.

Chen, X. L., D. B. Hausman, R. G. Dean, and G. J. Hausman. 1998. Hormonal regulation of leptin mRNA expression and preadipocyte recruitment and differentiation in porcine primary cultures of S-V cells. Obes. Res. 6:164–172.

Chen, X., D. B. Hausman, R. G. Dean, and G. J. Hausman. 1997. Differentiation-dependent expression of obese (ob) gene by preadipocytes and adipocytes in primary cultures of porcine stromal-vascular cells. Biochim. Biophys. Acta 1359:136–142.

Czaja, K., M. Lakomy, W. Sienkiewicz, J. Kaleczyc, Z. Pidsudko, C. R. Barb, G. B. Rampacek, and R. R. Kraeling. 2002. Distribution of neurons containing leptin receptors in the hypothalamus of the pig. Biochem. Biophys. Res. Commun. 298:333–337.

Elimam, A., A. C. Lindgren, S. Norgren, A. Kamel, C. Skwirut, P. Bang, and C. Marcus. 1999. Growth hormone treatment down-regulates serum leptin levels in children independent of changes in body mass index. Horm. Res. 52:66–72.

Etherton, T. D., E. D. Aberle, E. H. Thompson, and C. E. Allen. 1981. Effects of cell size and animal age on glucose metabolism in pig adipose tissue. J. Lipid Res. 22:72–80.

Fain, J. N., and S. W. Bahouth. 1998. Effect of tri-iodothyronine on leptin release and leptin mRNA accumulation in rat adipose tissue. Biochem. J. 332:361–366.

Fain, J. N., J. H. Ihle, and S. W. Bahouth. 1999. Stimulation of lipolysis but not of leptin release by growth hormone is abolished in adipose tissue from Stat5a and b knockout mice. Biochem. Biophys. Res. Commun. 263:201–205.

Florkowski, C. M., G. R. Collier, P. Z. Zimmet, J. H. Livesey, E. A. Espiner, and R. A. Donald. 1996. Low-dose growth hormone replacement lowers plasma leptin and fat stores without affecting body mass index in adults with growth hormone deficiency. Clin. Endocrinol. (Oxf.) 45:769–773.

Fors, H., H. Matsuoka, I. Bosaeus, S. Rosberg, K. A. Wikland, and R. Bjarnason. 1999. Serum leptin levels correlate with growth hormone secretion and body fat in children. J. Clin. Endocrinol. Metab. 84:3586–3590.

Gomez, J. M., F. J. Maravall, N. Gomez, M. A. Navarro, R. Casamitjana, and J. Soler. 2003. Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin. Endocrinol. (Oxf.) 58:213–219.

Gong, D.-W., S. Bi, R. E. Pratley, and B. D. Weintraub. 1996. Genomic structure and promoter analysis of the human obese gene. J. Biol. Chem. 271:3971–3974.

Guerre-Millo, M. 2002. Adipose tissue hormones. J. Endocrinol. Invest. 25:855–861.

Halleux, C. M., I. Servais, B. A. Reul, R. Detry, and S. M. Brichard. 1998. Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. J. Clin. Endocrinol. Metab. 83:902–910.

Harris, R. B. 2000. Leptin—Much more than a satiety signal. Annu. Rev. Nutr. 20:45–75.

Houseknecht, K. L., C. P. Portocarrero, S. Ji, R. Lemenager, and M. E. Spurlock. 2000. Growth hormone regulates leptin gene expression in bovine adipose tissue: Correlation with adipose IGF-1 expression. J. Endocrinol. 164:51–57.

Isozaki, O., T. Tsushima, M. Miyakawa, H. Demura, and H. Seki. 1999. Interaction between leptin and growth hormone (GH)/IGF-I axis. Endocr. J. 46(Suppl):S17–S24.

Joyce, C. 2002. Quantitative RT-PCR. A review of current methodologies. Methods Mol. Biol. 193:83–92.

Kanu, A., J. N. Fain, S. W. Bahouth, and G. S. Cowan, Jr. 2003. Regulation of leptin release by insulin, glucocorticoids, G(i)-coupled receptor agonists, and pertussis toxin in adipocytes and adipose tissue explants from obese humans in primary culture. Metabolism 52:60–66.

Kristensen, K., S. B. Pedersen, B. L. Langdahl, and B. Richelsen. 1999. Regulation of leptin by thyroid hormone in humans: studies in vivo and in vitro. Metabolism 48:1603–1607.

Lee, K. N., I. C. Jeong, S. J. Lee, S. H. Oh, and M. Y. Cho. 2001. Regulation of leptin gene expression by insulin and growth hormone in mouse adipocytes. Exp. Mol. Med. 33:234–239.

Leininger, M. T., C. P. Portocarrero, A. P. Schinckel, M. E. Spurlock, C. A. Bidwell, J. N. Nielsen, and K. L. Houseknecht. 2000. Physiological response to acute endotoxemia in swine: effect of genotype on energy metabolites and leptin. Domest. Anim. Endocrinol. 18:71–82.

Lin, J., C. R. Barb, R. R. Kraeling, and G. B. Rampacek. 2003. Growth hormone releasing factor decreases long form leptin receptor expression in porcine anterior pituitary cells. Domest. Anim. Endocrinol. 24:95–101.

Menendez, C., M. Lage, R. Peino, R. Baldelli, P. Concheiro, C. Dieguez, and F. F. Casanueva. 2001. Retinoic acid and vitamin D(3) powerfully inhibit in vitro leptin secretion by human adipose tissue. J. Endocrinol. 170:425–431.

Mersmann, H. J. 1989. The effect of insulin on porcine adipose tissue lipogenesis. Comp. Biochem. Physiol. B 94:709–713.

Morash, B., J. Johnstone, C. Leopold, A. Li, P. Murphy, E. Ur, and M. Wilkinson. 2000. The regulation of leptin gene expression in the C6 glioblastoma cell line. Mol. Cell. Endocrinol. 165:97–105.

Mueller, W. M., F. M. Gregoire, K. L. Stanhope, C. V. Mobbs, T. M. Mizuno, C. H. Warden, J. S. Stern, and P. J. Havel. 1998. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 139:551–558.

Murakami, T., M. Iida, and K. Shima. 1995. Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem. Biophys. Res. Commun. 214:1260–1267.

O'Connell, J. 2002. The basics of RT-PCR. Some practical considerations. Methods Mol. Biol. 193:19–25.

Ramsay, T. G., I. B. Chung, S. M. Czerwinski, J. P. McMurtry, R. W. Rosebrough, and N. C. Steele. 1995. Tissue IGF-I protein and mRNA responses to a single injection of somatotropin. Am. J. Physiol. Endocrinol. Metab. 269:E627–E635.

Ramsay, T. G., and M. E. White. 2000. Insulin regulation of leptin expression in streptozotocin diabetic pigs. J. Anim. Sci. 78:1497–1503.

Ramsay, T. G., X. Yan, and C. Morrison. 1998. The obesity gene in swine: sequence and expression of porcine leptin. J. Anim. Sci. 76:484–490.

Raver, N., E. E. Gussakovsky, D. H. Keisler, R. Krishna, J. Mistry, and A. Gertler. 2000. Preparation of recombinant bovine, porcine, and porcine W4R/R5K leptins and comparison of their activity and immunoreactivity with ovine, chicken, and human leptins. Protein Expr. Purif. 19:30–40.

Richards, M. P., and S. M. Poch. 2002. Quantitative analysis of gene expression by reverse transcription polymerase chain reaction and capillary electrophoresis with laser-induced fluorescence detection. Mol. Biotechnol. 21:19–37.

Russell, C. D., R. N. Petersen, S. P. Rao, M. R. Ricci, A. Prasad, Y. Zhang, R. E. Brolin, and S. K. Fried. 1998. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am. J. Physiol. Endocrinol. Metab. 275:E507–E515.

Slieker, L. J., K. W. Sloop, P. L. Surface, A. Kriauciunas, F. LaQuier, J. Manetta, J. Bue-Valleskey, and T. W. Stephens. 1996. Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. J. Biol. Chem. 271:5301–5304.

Smith, J. T., and B. J. Waddell. 2002. Leptin receptor expression in the rat placenta: changes in ob-ra, ob-rb, and ob-re with gestational age and suppression by glucocorticoids. Biol. Reprod. 67:1204–1210.

Spurlock, M. E., M. A. Ranalletta, S. G. Cornelius, G. R. Frank, G. M. Willis, S. Ji, A. L. Grant, and C. A. Bidwell. 1998. Leptin expression in porcine adipose tissue is not increased by endotoxin but is reduced by growth hormone. J. Interferon Cytokine Res. 18:1051–1058.

Walton, P. E., and T. D. Etherton. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: Antagonism by porcine growth hormone. J. Anim. Sci. 62:1584–1595.

Wang, J., R. Liu, L. Liu, R. Chowdhury, N. Barzilai, J. Tan, and L. Rossetti. 1999. The effect of leptin on Lep expression is tissue-specific and nutritionally regulated. Nat. Med. 5:895–899.

Van Harmelen, V., S. Reynisdottir, P. Eriksson, A. Thorne, J. Hoffstedt, F. Lonnqvist, and P. Arner. 1998. Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 47:913–917.

Yoshida, T., T. Monkawa, M. Hayashi, and T. Saruta. 1997. Regulation of expression of leptin mRNA and secretion of leptin by thyroid hormone in 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 232:822–826.